

## Deep Sedation and Mechanical Ventilation without Paralysis for 3 Weeks in Normal Beagles

### Exaggerated Resistance to Metocurine in Gastrocnemius Muscle

Gerald A. Gronert, M.D.,\* Dennis L. Fung, M.D.,\* Steve C. Haskins, D.V.M.,† Eugene P. Steffey, V.M.D., Ph.D.†

**Background:** Patients in the intensive care unit may have muscle weakness in the recovery phase, and disuse atrophy may play a role in this weakness. To assess this problem, the authors measured changes in the potency of the nondepolarizing neuromuscular blocking agent metocurine in a canine model that involved 3 weeks of intensive care, nonparalyzing anesthesia with pentobarbital, and positive-pressure ventilation.

**Methods:** Six dogs were anesthetized with pentobarbital to a sufficient depth that spontaneous and reflex muscle movements were absent. Their tracheas were intubated, their lungs were mechanically ventilated, and they received round-the-clock intensive medical and nursing care for 3 weeks. Transduced gastrocnemius muscle responses to metocurine were determined weekly. A 4- to 15-min infusion of 148–4,300  $\mu\text{g}/\text{min}$  (longer durations and greater concentrations on progressive weeks) yielded more than 80% paralysis. Serial metocurine plasma concentrations during the onset of the block and recovery provided data to determine pharmacokinetics using NONMEM. Metocurine plasma concentrations and the degree of paralysis were used to model the effect compartment equilibration constant, and the Hill equation was used to yield the slope factor and potency within the effect compartment.

**Results:** The metocurine effect compartment concentration associated with a 50% diminution of twitch height after 3 weeks was  $1,716 \pm 1,208$  ng/ml (mean  $\pm$  SD), which was significantly different from  $257 \pm 34$  ng/ml, the value on day 0. There were no pharmacokinetic differences.

**Conclusion:** The absence of muscle tone and reflex responsiveness for 3 weeks was associated with exaggerated resistance to the neuromuscular blocker metocurine. (Key words: Disuse atrophy; intensive care unit; pharmacodynamics; pharmacokinetics; skeletal muscle neuromuscular blocking agents.)

\* Department of Anesthesiology, School of Medicine.

† Department of Surgical and Radiological Sciences, School of Veterinary Medicine.

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Address reprint requests to Dr. Gronert: Department of Anesthesiology, TB 170, University of California, Davis, Davis, California 95616. Address electronic mail to: ggronert@ucdavis.edu

RECOVERY of muscle use and strength tends to be delayed in patients undergoing prolonged intensive care.<sup>1</sup> This may result, in part, from bed rest-related atrophy of disuse in the absence of evident nerve damage. Therefore, we designed an intensive care canine model likely to involve disuse atrophy and its associated resistance to blockade by nondepolarizing neuromuscular blocking agents.<sup>2</sup> Our theory was that the gastrocnemius  $\text{IC}_{50}$  (effect compartment concentration associated with 50% diminution of twitch height) for metocurine would increase markedly during a 3-week period of absolute immobility in an intensive care unit (ICU) environment.

### Materials and Methods

Eight Beagles began this 3-week study, which was approved by our animal use and care committee. Dogs were cared for as previously described.<sup>3</sup> They were sedated deeply with pentobarbital, initially at a dosage of 5 to 6  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ , to a depth that eliminated spontaneous muscle movement, including respiration and shivering. Their tracheas were intubated (with an endotracheal tube change every 3 days), and mechanical ventilation was initiated. Ventilation was started with inspired oxygen, 21%, at a tidal volume of 15 ml/kg, 15 breaths/min, and adjusted as necessary to maintain the partial pressure of carbon dioxide in arterial blood ( $\text{Pa}_{\text{CO}_2}$ ) at 35–42 mmHg, the partial pressure of oxygen ( $\text{Pa}_{\text{O}_2}$ ) greater than 80 mmHg, and pulse oximeter saturation (shaved tail) greater than 95%. The depth of anesthesia sedation was evaluated by loss of palpebral reflex, mandibular and appendicular muscle tone, and spontaneous movement or shivering, and by changes in eye position, blood pressure, and heart rate. Maintenance care included intravenous fluids, gastric feedings, a change of position every 4 h (left lateral, sternal, right lateral, and dorsal, an animal term identical to *supine* in



humans), tracheal suction, manual expression of urine, and other necessary ICU procedures. Peripheral intravenous and femoral arterial catheters were placed aseptically and changed every 7 days, earlier if there was inflammation. The antibiotic ticarcillin clavulanate (no interaction with neuromuscular blockers) was added if there were signs of infection. Care was provided constantly 24 h/day.<sup>3</sup>

Metocurine concentration response studies were performed weekly to measure potency in producing gastrocnemius skeletal muscle paralysis. The legs of the supine dog were fastened with straps and tape in a rigid padded frame, with ankles, knees, and hips fixed at right angles. The strength of unilateral plantar flexion was measured using a force transducer (FT 10; Grass Instruments, Braintree, MA). The sciatic nerve was stimulated by surgically implanted (aseptic technique) electrodes, each 30 s, with a train-of-four (2 Hz for 2 s; duration, 0.1 s) using a constant-current stimulator (model 6i; Haer Instruments, Brunswick, ME) at twice the supramaximal current (6–10 mA). Transducer output was recorded by penwriter, digitized (model 2821; Data Translations, Marlboro, MA) and analyzed in real time using a customized program. The signal from each contraction in the train-of-four was stored as 1,000 points acquired at 2-ms intervals and analyzed for resting tension and peak contractile response of the first and fourth twitches. The optimal length of the gastrocnemius muscle was determined by rotating a foot pedal; its point of contact, the beam of the transducer, was the tarsus just proximal to the foot pad. The fulcrum was colinear with the point of ankle flexion. The foot pedal was advanced at 3-degree intervals until the maximal twitch amplitude was obtained.

The metocurine infusion was begun when vital signs were stable and twitch amplitude did not vary more than 3% for 15 min. Indicators of stability included systolic blood pressure of 100–160 mmHg and diastolic pressure of 60–100 mmHg; heart rate, 90–150 beats/min; rectal temperature, 37–39°C, maintained by a circulating water mattress and a heat lamp; pulse oximeter value, more than 95%; PaO<sub>2</sub>, 80–100 mmHg while breathing oxygen, 21%, supplemented with added inspired oxygen if the PaO<sub>2</sub> was less than 80 mmHg; end-expired carbon dioxide, 32–38 mmHg; and PaCO<sub>2</sub>, 35–42 mmHg. Metocurine was infused for 4–15 min at 148–4,300 µg/min (with a longer duration and greater concentration in progressive weeks) until it produced more than 80% diminution of the first twitch in the train-of-four. Three-milliliter arterial blood samples for the metocurine assay were drawn

at 0, 1.5, 3, 4.5, 6, 7.5, 9, 10.5, 12, 13.5, 15, 16.5, 18, 20, 25, 30, 45, 60, 90, 120, 150, 180, and 240 min. Sciatic nerve stimulation was halted (always within 240 min) when the muscle twitch amplitude had recovered completely and the train-of-four ratio was at least 0.75. Our laboratory determined the plasma concentration of metocurine by high-performance liquid chromatography.<sup>4</sup>

The computer program NONMEM (Drs. L. Sheiner and S. Beal, Laboratory Medicine, UCSF, San Francisco, CA) used time and plasma concentration data and nonlinear mixed-effects modeling to determine the metocurine population pharmacokinetics.<sup>5</sup> A two-compartment model provided the best fit to the data. A semiparametric analysis of time, plasma concentration, and degree of paralysis estimated the equilibration constant between the effect compartment and the plasma (K<sub>e0</sub>).<sup>6</sup> The Hill equation determined the IC<sub>50</sub> and the slope factor  $\gamma$  as follows:

$$\text{Effect} = \frac{1.0 \times \text{Ce}^\gamma}{\text{IC}_{50}^\gamma + \text{Ce}^\gamma}$$

where Effect is the degree of paralysis, 1.0 is the maximum effect or 100% blockade, Ce is the effect site concentration, IC<sub>50</sub> is the effect site concentration associated with 50% diminution of the twitch, and  $\gamma$  is the slope factor.<sup>4</sup>

#### Statistical Analyses

Data are reported as the mean  $\pm$  SD. We used the logarithmic mean for analysis of kinetic data, in part because it reduces the influence of larger outliers,<sup>5</sup> and repeated measures analysis of variance to detect differences among the various weeks. The Dunnett multiple comparisons test was used to confirm differences ( $P < 0.05$ ).

#### Results

Two dogs died, one suddenly on day 2 because of an apparent air embolism. The other had aspiration pneumonia when the gastric feeding tube was not placed completely into the stomach; this dog died on day 6. Neither dog was included in the calculations of pharmacokinetics or pharmacodynamics. The remaining six dogs survived the 3-week period of study in good health, without weight loss (table 1), and with stable and normal vital signs throughout, similar to the dogs included in our previous study.<sup>3</sup> The rate of pentobarbital infusion, initially 5 to 6 mg  $\cdot$  kg<sup>-1</sup>  $\cdot$  h<sup>-1</sup>, was doubled by the end



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Table 1. Pharmacodynamics during Beagle ICU Care

Day	$K_{e0}$ ( $\text{min}^{-1}$ )	$\text{IC}_{50}$ (ng/ml)	$\gamma$	Weight (kg)
0	$0.243 \pm 0.0759$	$247 \pm 34$	$6.0 \pm 2.0$	$9.6 \pm 4.0$
7	$0.230 \pm 0.0842$	$436 \pm 96$	$4.9 \pm 1.7$	$10.2 \pm 4.3$
14	$0.115 \pm 0.0402^{*†}$	$991 \pm 571$	$3.1 \pm 1.8^{*}$	$10.0 \pm 3.9$
21	$0.103 \pm 0.0462^{*†}$	$1,716 \pm 1,208^{*†}$	$2.0 \pm 1.0^{*†}$	$9.3 \pm 4.3$

Values are mean  $\pm$  SD; N = 6.

$K_{e0}$  = equilibration constant for metocurine;  $\text{IC}_{50}$  = effect site metocurine concentration at which there is 50% twitch diminution;  $\gamma$  = slope factor of the sigmoidal concentration response curve.

\* Different from day zero ( $P < 0.05$ ).

† Different from day 7 ( $P < 0.05$ ).

of the third week (individual data available but not reported).

Mean pharmacokinetic values (table 2) did not change during the 3-week study. As for the pharmacodynamic findings (table 1),  $K_{e0}$  and  $\gamma$  decreased significantly by days 14 and 21; the  $\text{IC}_{50}$  (table 1, fig. 1) increased from day 0 to day 21.

## Discussion

These results extend our work with a long-term model involving dogs that were anesthetized with pentobarbital and mechanically ventilated,<sup>3</sup> and they characterize the alterations in responses to the nondepolarizing skeletal muscle neuromuscular blocking agent metocurine during an extended period of apparent complete muscle disuse. We had theorized that the total lack of neuromuscular activity would result in greater changes in response to a neuromuscular blocking agent than our previous model involving cast immobilization.<sup>7</sup> Our findings support our hypothesis.

A 3-week period of hind limb cast immobilization had resulted in a tripling of the  $\text{IC}_{50}$  of metocurine, from 262 ng/ml to 735 ng/ml.<sup>7</sup> In the current study, after 3 weeks' sedation and mechanical ventilation, the  $\text{IC}_{50}$  for metocurine increased from 257 ng/ml to 1,716 ng/ml. This implies that complete and total inactivity is associated with greater changes in neuromuscular function. The ICU model resulted in greater inactivity of skeletal muscle, with likely greater muscle atrophy. The associated diminished release of acetylcholine led to receptor up-regulation (development of extrajunctional acetylcholine receptors), which would explain the resistance to metocurine.<sup>2</sup> This greater degree of resistance also probably occurred, in addition to that in the gastrocnemius muscle, in the rest of the body musculature, although we

do not have data to confirm this. Some of this change may have been related to decreases in cholinesterase activity associated with disuse atrophy.<sup>8</sup> Our casted hind limb model of immobilization yields significant gastrocnemius atrophy (40% loss of weight in 4 weeks, similar to that observed in sciatic denervation),<sup>9</sup> and we believe that the ICU model is at least as effective in that regard. Alterations in renal function do not account for these findings: Although metocurine is excreted primarily by the kidney, renal function is not altered in this model.<sup>3</sup> We tried to measure the gastrocnemius muscle acetylcholine receptor concentration in this ICU study, but various difficulties limited our assay to the intercostal muscle in three of the dogs.<sup>10</sup> Based on a technique involving a high-titer polyclonal antiserum, the average nicotinic receptor concentration was  $1.40 \pm 0.84$  mol/g tissue at 21 days (normal values, 0.2–0.4 pmol/g).<sup>10</sup>

Resistance to a nondepolarizing neuromuscular blocking agent in these studies appears to be related to a prolonged period of diminished acetylcholine release during sustained disuse, and, in the current study, perhaps additionally to a large "sink" of up-regulated skeletal muscle throughout the body that has undergone up-regulation and therefore binds additional molecules of metocurine.<sup>2</sup> Figure 2 illustrates the relation of muscle use and potency, a compilation from this and previous studies. There is a progression of metocurine potency: It is most potent in a canine exercise model<sup>11</sup>, followed by a normal, then a limited, disuse model (cast immobilization)<sup>7</sup>, and it is least potent in our ICU model. Data from these studies may be compared because the protocols and species were similar: For the exercise study,<sup>11</sup> dogs received pentobarbital and nitrous oxide; for the cast immobilization disuse study,<sup>7</sup> dogs received thiamylal, fentanyl (0.1–0.4 mg), and nitrous oxide; and for the current study dogs received only pentobarbital. The curve for the normal gastrocnemius muscle (fig. 2) represents a compilation of 28 normal values from all of these studies: nonexercised dogs<sup>11</sup>; dogs that had cast immobilization disuse on day 0, with data from both legs before the casts were applied<sup>7</sup>; and day 0 data from the current study.

We had originally selected metocurine for our first pharmacodynamic study of disuse because it had a longer duration and was metabolized slowly; modeling (at that time) was thus easier, and a practical assay was available.<sup>12</sup> We continued to use metocurine because it provided a means to compare various muscle use potency situations in the same species and using the same neuromuscular blocking agent.



Table 2. Pharmacokinetics during Beagle ICU Care

Day	Central Volume (l/kg)	Vd <sub>ss</sub> (l/kg)	Clearance Distributional (ml · kg <sup>-1</sup> · min <sup>-1</sup> )	Clearance Elimination (ml · kg <sup>-1</sup> · min <sup>-1</sup> )	T <sub>1/2α</sub> (min)	T <sub>1/2β</sub> (min)
0	0.0702 ± 0.0247	0.2679 ± 0.0913	2.63 ± 1.16	10.3 ± 4.8	3.6	87.7
7	0.1242 ± 0.1223	0.3326 ± 0.1573	2.98 ± 0.48	8.64 ± 1.48	1.8	25.3
14	0.0824 ± 0.0254	0.3049 ± 0.046	3.03 ± 0.51	9.46 ± 3.02	5.2	86.8
21	0.0987 ± 0.0529	0.346 ± 0.177	3.74 ± 0.98	10.75 ± 6.75	3.9	24.5
					4.1	84.6
					2.1	15.1
					3.9	75.2
					0.9	19.2

Values are mean ± SD; N = 6. There were no significant differences among kinetic values.

Vd<sub>ss</sub> = volume of distribution at steady state.

The changes in  $K_{e0}$  by days 14 and 21 (table 1) reflect a doubling of the half-time for equilibration at the effect site for metocurine at a time when potency had decreased. These changes do not support the proposal that there is an inverse relation between potency and onset.<sup>13</sup> The factors governing  $K_{e0}$  appear to involve more than potency: there may be additional perijunctional receptors,  $K_{e0}$  may depend in part on muscle perfusion, and it may depend in part on the effect site concentration of metocurine, which is increased as the potency decreases. This ICU-related change in  $K_{e0}$  was not seen in our study of cast immobilization disuse<sup>7</sup>; differing factors include a smaller change in potency, the lack of a prolonged period of anesthesia, and a regular diet. The effects of prolonged anesthesia in this regard may have included altered peripheral circulation, altered fluid balance in skeletal muscle, and depression of reactions by accumulated barbiturate.

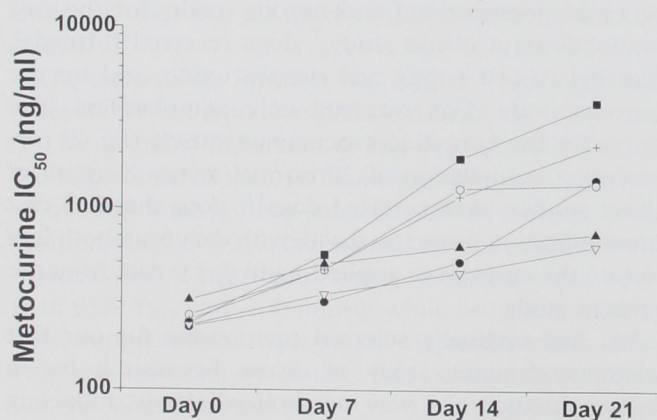


Fig. 1. The canine gastrocnemius muscle effect compartment concentration associated with a 50% diminution of the twitch for metocurine in individual dogs versus the day of intensive care unit care during 3 weeks of whole-body total disuse atrophy in an intensive care unit environment. See table 1 and the text for details.

Body weight was stable during this study, despite what we perceived as responses related to diminished muscle mass (muscle atrophy in this preparation was previously confirmed in our succinylcholine study of the casted hind limb<sup>9</sup>). It is possible that the dogs had developed some generalized edema; there may have been fluid retention, perhaps related to stress and the renin-angiotensin-aldosterone system. We did not perform a morphometric analysis. Finally, the mortality rate was decreased from 47% in our previous study<sup>3</sup> to 25%.

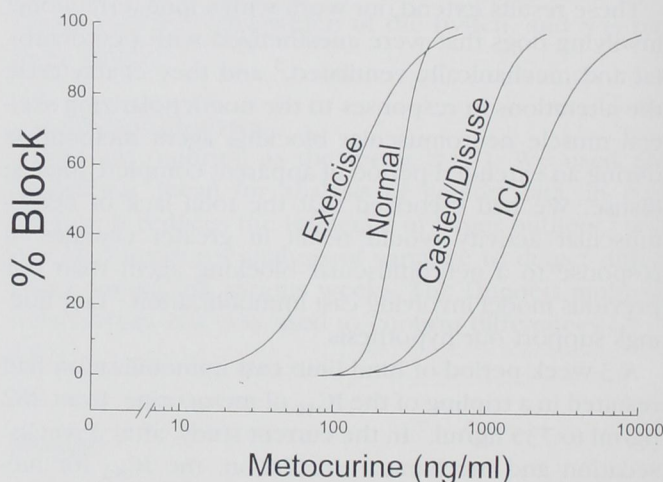


Fig. 2. Canine gastrocnemius muscle paralysis versus the effect site concentration of metocurine among dogs with long-term conditioning exercise, healthy dogs, and dogs with either hind limb cast immobilization or disuse atrophy instituted by deep sedation in the intensive care unit. The concentration associated with 50% diminution of the twitch ( $IC_{50}$ ) for each study differs from its pretreatment normal value. Data are from the current study and from work by Fung *et al.*<sup>7</sup> and Gronert *et al.*<sup>11</sup> The curve from healthy dogs represents pooled control values taken at the beginning of each study (see text for details). Mean metocurine  $IC_{50}$  values and slope factors used to generate these curves include exercise: 114 ng/ml,  $\gamma$  2; normal: 255 ng/ml,  $\gamma$  5.6; casted hind limb disuse: 735 ng/ml,  $\gamma$  5.1; and intensive care unit disuse: 1,716 ng/ml,  $\gamma$  2.



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This straightforward but complex model is based on underlying hypotheses related to physiology (muscle use governs the functional number of acetylcholine receptors), clinical medicine (muscle disuse leads to resistance to nondepolarizing neuromuscular blocking agents), and basic science (acetylcholine receptor numbers are regulated through a feedback mechanism currently not understood). Furthermore, this model may provide a means to evaluate in healthy animals some of the various alterations induced by an ICU environment that are related to ICU care *per se* rather than to a primary disease.

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